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The urinary corticoid:creatinine ratio (UCCR) in healthy cats undergoing hospitalisation

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Thirty-one healthy pet cats had voided urine samples collected prior to, during and after a brief period of hospitalisation. Urinary corticoids were measured, both prior to and following an extraction technique, and the urinary corticoid:creatinine ratio (UCCR) was calculated. Associations between the UCCR and age, sex, breed and time of urine collection were investigated. There was no significant relationship established between age, sex and breed and the UCCR. A significant increase in the UCCR, however, did occur between the first home collected and first hospitalised urine sample, but only when comparing extracted corticoid results. A normal range for feline UCCR is established for the chemiluminescent immunoassay used in this study.

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Introduction

Urinary corticoids have been used as an indicator of hypothalamic-pituitary-adrenal (HPA) axis activity in a variety of animal species such as horses (Chandler and Dixon 2002), pigs (Pol et al 2002), dogs (van Vonderen et al 1998), and in both non-domestic (Carlstead et al 1992) and domestic cats (Carlstead et al 1993, Rochlitz et al 1998). Urinary corticoid levels accurately reflect changes in total plasma cortisol over the preceding period of urine production, tending to reflect an averaged estimate of the cortisol release that occurs both in healthy and diseased (Jones et al 1990, Kemppainen and Peterson 1996). When corrected for urine concentration by expressing urinary corticoids as a urine corticoid:creatinine ratio (UCCR), the ratio provides a quantitative assessment of cortisol release over a variable time period predominantly determined by the previous micturition pattern.

In cats, although cortisol is the primary glucocorticoid released by the adrenal gland only a small amount of the free cortisol, cortisol metabolites and conjugates produced are renally excreted (Goossens et al 1995, Graham and Brown 1996). Despite this, the UCCR has shown promise in measuring the activity of the feline HPA axis (Goossens et al 1995).

It is well accepted that various stressors such as unfamiliar handling, skin testing and changes in husbandry are potent activators of the feline HPA. These stressors increase both serum cortisol concentration and the UCCR in cats (Carlstead et al 1993, Willemse et al 1993, Rochlitz et al 1998), and so it was hypothesised that the UCCR would increase following confinement in the hospital. This effect has been documented in dogs undergoing hospitalisation and occurs to such an extent that it can interfere with diagnostic testing (van Vonderen et al 1998).

Normal values for the feline UCCR in healthy pet cats have been published based on a small number of cats in a hospital environment (Henry et al 1996) and in a different study, based on a population of cats in their home environment (Goossens et al 1995). Furthermore, it has been shown in a small group of quarantine cats that the UCCR rises then falls rapidly after entering the cattery, returning to within the normal range by the second day of confinement (Rochlitz et al 1998). However, no study has evaluated changes in UCCR estimated in voided urine from a normal cat population where the cats were first sampled at home and then following transport to, and hospitalisation in, a standard veterinary practice.

Additionally, in cats it remains unclear whether urine samples need to undergo an extraction procedure prior to assay for cortisol in order to 'free'

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the cortisol from interferents such as the glucuronide and sulphate conjugates of cortisol and its metabolites. These products have been shown to produce positive interference in a number of immuno-assay systems (Rijnberk et al 1988) in species excreting larger proportions of their urinary corticosteroids as free cortisol. Since cats excrete much less (<2%) it is possible that the analysis of UCCR in cats may be even more prone to positive interference from these interferents.

The aims of this study were to initially investigate if urine extraction in cats is needed to obtain meaningful UCCR results and at the same time establish the stability of urinary corticoids in urine stored under different conditions. Once a reliable and appropriate method of measuring UCCR in feline urine had been established, this methodology could then be applied to establish a normal range for the UCCR in healthy cats in their home environment and quantify any changes in the UCCR once these same healthy cats were subjected to unfamiliar handling and hospitalisation.

Material and methods

Urine collection

Healthy pet cats belonging to members of hospital staff, veterinary students and clinic clients were recruited for the study. All underwent a full physical examination and a health screen that included a haematological and biochemical panel as well as serum thyroxine levels on the first day of hospitalisation. Chemical urinalysis and urine specific gravity were measured on at least one occasion. Cats were included if no clinically significant abnormalities could be found on these examinations.

Four free catch urine samples were collected at different times from each of the cats using KatKor non-absorbent cat litter (Rein Vet Products, Holland). Voided urine was collected from the litter tray with a plastic pipette and stored in plastic containers. The first sample (time 1) was collected at home within 7 days of presentation to a veterinary hospital. The second sample (time 2) was the first urine sample produced after admission and the third sample (time 3) was that produced on day 3 or later. Due to the inconsistent urination habits of hospitalised cats these second and third urine samples were not necessarily produced on day 1 and day 3. There was no limit given to the time spent in the hospital, but the longest stay was 6 days in order to collect the two samples. The final urine sample (time 4) was collected at home between 2 and 10 days after discharge from the hospital. All cats were housed in individual cages as per routine clinical cases. Urine on collection was frozen as soon as possible at −20°C and batched prior to analysis.

Assessment of urine corticoid stability under different urine storage conditions

Five urine samples (two samples from the study population and three from sick cats) were stored under different conditions to assess the stability of urinary corticoids based on samples collected with KatKor litter. Each of the five urine samples was divided into five aliquots and each was treated in one of the following ways: (i) immediate freezing, (ii) collection into a vial and leaving at room temperature for 24 h prior to freezing, (iii) collection then chilling (4°C) for 24 h prior to freezing, (iv) leaving at room temperature in litter for 24 h then freezing, and (v) putting the urine through a single cycle of freeze–thawing before final refreezing. Urine corticoids were measured prior and following extraction on all samples and the UCCR calculated.

Analysis of samples

Haematology and serum biochemistry were analysed by the commercial laboratory at the Royal Veterinary College (RVC) using standard techniques. Serum total thyroxine was measured by a competitive chemiluminescent immunoassay (Diagnostic Products Corporation (DPC), Immulite) and urine creatinine concentration was measured using the Jaffé method in an automated biochemistry analyser (Bayer Opera). Urinary corticoids were measured on both extracted and non-extracted samples. Extraction was performed on one aliquot using dichloromethane, according to a standardised extraction procedure (Lamb et al 1994). Both aliquots were then analysed by a competitive chemiluminescent immunoassay (DPC, Immulite), as per the serum cortisol assay. Urine quality control material was run alongside the usual serum quality controls. Assay precision was assessed using pooled urine and commercial quality control material.

Statistical analysis

The data were analysed using the PC compatible SPSS (release 10.0) computer package. Reference ranges for the UCCR were calculated as the mean±1.96 standard deviation. To address the

affect of time of urine collection, age, breed and sex on the UCCR two separate repeated measures analyses of variance (ANOVA) were conducted, one with the dependent variable being extracted UCCR and the other dependent variable being non-extracted UCCR. For both analyses, the independent variables were time of sample collection, sex, age and breed. The cats' ages were divided into quartiles and evaluated as a categorical variable. Where a statistically significant effect of an independent variable was found, the Holm's sequential Bonferroni procedure was used to conduct pairwise comparisons with a controlled error rate across tests at the 0.05 level.

In the samples stored under different conditions to assess corticoid stability, coefficient of variation (CV) values were calculated for the UCCRs. In addition, a non-parametric ANOVA (Friedman's test) was used to look for bias between the storage conditions.

Results

Thirty-one cats satisfied the criteria for inclusion. These ranged in age from 1 to 15 years (median 5.7 years). Thirteen were neutered females and 18 were neutered males. Twenty-four cats were domestic short or long hair cats and seven were pedigree (two Persians, two Burmese, three Birmans). The three sick cats whose urine was collected to assess urine corticoid stability were the cats hospitalised for a variety of injuries and illnesses.

The Immulite assay gave a range for urine corticoids from 27.6 to 1380 nmol/l. Low levels were reported as <27.6 nmol/l and a figure of 27.6 was used for samples measuring <27.6 nmol/l when subsequently calculating the UCCR. Intra-assay CVs were 17.7% at a mean of 62.4 nmol/l (extracted), and 4.4% at a mean of 1141.2 nmol/l (non-extracted). Inter-assay CVs were 10.1% at a mean of 52.4 nmol/l (extracted) and 7.2% at a mean of 168.9 nmol/l (non-extracted).

The urine samples assayed to address urine corticoid stability under different storage conditions showed CVs between 6.11 and 19.3% for extracted samples and between 2.65 and 13.4% for non-extracted urine. Obvious bias was not shown using the Friedman's test (extracted UCCRs, $p=0.55$, non-extracted UCCRs, $p=0.16$). Katkor non-absorbable litter was considered suitable for voided urine collection and urine corticoids appeared stable even when kept at room temperature for 24 h. The higher variation in the UCCR that was seen in the extracted samples may reflect the additional steps required in performing the extraction and the introduction of additional error during repeated handling of the samples.

Table 1. Urine corticoid:creatinine ratio (UCCR) at different collection times from extracted urine corticoid (UCCRe), and non-extracted urine corticoid (UCCRne), measurements

UCCR	Mean±SD	Range	Reference range
UCCRe, time 1	2.90±1.96	1.27–10.59	0–6.74
UCCRe, time 2	4.15±1.59	1.8–10.27	1.03–7.27
UCCRe, time 3	3.35±1.27	1.31–6.93	0.86–5.84
UCCRe, time 4	3.20±1.34	1.18–6.47	0.57–5.83
UCCRne, time 1	4.77±3.03	1.91–16.61	0–10.71
UCCRne, time 2	6.28±2.66	2.76–17.43	1.07–11.49
UCCRne, time 3	5.50±2.18	1.61–11.15	1.23–9.77
UCCRne, time 4	4.75±2.00	1.68–10.09	0.83–8.67

The reference range was calculated as the mean±1.96 SD.

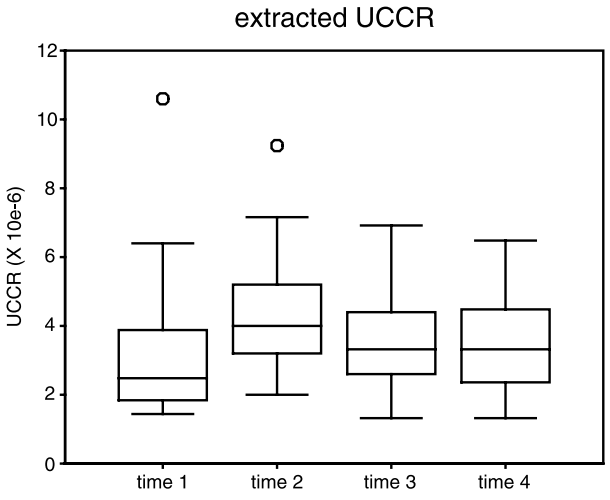

Fig 1. Box–whisker plot of extracted UCCR at the four different collection times. The boxes represent the 25th and 75th percentiles and the horizontal line the median. The whiskers encompass the bulk of the data, excluding outliers, and in most cases represent the range. Outliers are shown as circles. A significant difference was present between time 1 and time 2 ($p<0.001$), and between time 2 and time 4 ($p=0.010$).

The means±standard deviation and ranges of the UCCR, for extracted and non-extracted urine, at different collection times in healthy cats are shown in Table 1 together with calculated reference ranges. A box–whisker plot of extracted UCCR at the different collection times is shown in Fig 1 and for non-extracted UCCR in Fig 2.

The analysis of the extracted UCCR results indicated a significant time effect, $F=4.547$, $p=0.008$. Follow-up pairwise comparisons indicated that 'time 1' was significantly different from 'time 2', ($p<0.01$) and 'time 2' was significantly different from 'time 4', ($p=0.010$). No

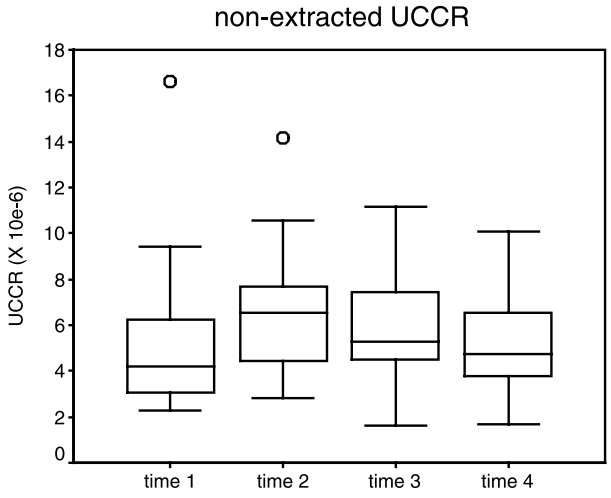

Fig 2. Box–whisker plot of non-extracted UCCR at the four different collection times. The boxes represent the 25th and 75th percentiles and the horizontal line the median. The whiskers encompass the bulk of the data, excluding outliers, and in most cases represent the range. Outliers are shown as circles, ○. No significant difference was found between the different collection times.

other significant differences between collection times were found nor there was any correlation found between the extracted UCCR values and sex (p=0.482), breed (p=0.128) or age (p=0.074). For the analysis of non-extracted UCCR values, time was not found to significantly affect outcome, p=0.118 and no significant effect on the UCCR was found due to sex (p=0.755), breed (p=0.793) or age (p=0.939).

Discussion

This study demonstrates that when cats are hospitalised and/or subjected to routine veterinary procedures there are significant increases in their extracted UCCR. This is not surprising as changes in routine and handling are potent psychological stressors known to activate the HPA. A significant increase in the UCCR in the hospital environment was only documented for extracted urine samples and this may be a reflection of the low proportion of corticoids excreted into cat urine. Total urinary corticoids, including the cortisol conjugates and metabolites, account for approximately 14% of total corticoid excretion in cats (Graham and Brown 1996) and additionally not all of these will be measured by the cortisol immunoassay. Consequently, subtle changes in free cortisol excretion may not be apparent on non-extracted samples. It may well be the case that once this immunoassay is used in cases of sick cats and in particular those with adrenal illness these subtleties may not be of concern and then non-extracted samples may be considered adequate.

The ranges of the UCCR in our study were lower than previously reported (Goossens et al 1995, Henry et al 1996). This discrepancy is likely to be in part due to differing methods of cortisol assay. It is important not to compare ranges using different assay methods, eg with and without prior extraction procedures, a conclusion shared by other authors (Smiley and Peterson 1993). Ranges need to be established for each assay and laboratory reflecting different antibody affinities and specificities, and laboratory techniques. A second explanation for the lower ranges is that the cats in this study were 'less stressed' than those in other reports. This may have arisen as many of the cats were hospitalised in clinics where their owners worked and it has been suggested that some cats actually enjoy the additional attention that handling and even blood collection entails (Carlstead et al 1992).

The study further indicated that urinary corticoids are stable under tested storage conditions and that the Katkor non-absorbable cat litter can be used to collect voided urine for the purpose of corticoid assay. This would make home collection viable and indeed preferable in the light of the changes in the UCCR documented.

Age, breed and gender failed to significantly influence the UCCR in this study. Pedigree cat numbers were low and this may have influenced the statistics, but there is as yet no known difference in HPA activity between differing cat breeds. This situation is not shared by other domestic species such as pigs where certain breeds have been shown to produce significantly more glucocorticoids (Hay and Mormède 1998). Previously it has been shown that as cats (Goossens et al 1995) and dogs (Rothuizen et al 1993) age the urinary corticoid excretion increases, but this could neither be corroborated by this study nor was it found in another study of healthy cats (Henry et al 1996).

It is hoped that by setting reliable reference ranges ultimately the UCCR may provide a useful screening test for feline hyperadrenocorticism. This is a rare disease in cats (Behrend and Kemppainen 2001) yet occurs with some regularity in other domestic species. The UCCR has been shown in dogs (Rijnberk et al 1988, Feldman and Mack 1992, Smiley and Peterson 1993, Den Hertog et al 1999), horses (van der Kolk et al 1995, Chandler and Dixon 2002) and ferrets (Gould et al 1995) to have a high negative predictive value and so makes it a useful screening

test for hyperadrenocorticism. Lack of specificity arises due to the effect on the HPA of non-adrenal illness and psychological stressors. Hospitalisation of dogs can elevate the UCCR sufficiently to yield results in the diagnostic range for hyperadrenocorticism (van Vonderen et al 1998). In cats, the UCCR also offers the possibility of providing a simple and convenient screening test for hyperadrenocorticism. However, in the small number of hyperadrenocorticoid cats investigated with the UCCR there was significant overlap between values obtained in the affected cats and in those that were considered normal (Goossens et al 1995). Current adrenal function tests used regularly in dogs are not well validated in cats (Behrend and Kemppainen 2001) and a single urine test is cheap and less invasive, but in light of the changes documented in this study the recommendation would be to collect the urine at home and use an extraction method before measuring urine corticoids.

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